Direction Leaflet Number Six

HOW TO PRESERVE A COLLECTION OF SOFT-BODIED INSECTS AND SPIDERS

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Although most adult insects are preserved by air-drying only, the general collector will find, among his catch, many interesting creatures which cannot be so treated without intolerable distortion. Almost all spiders, ticks, and daddy-long-legs, for instance, are so thin-skinned and succulent that, if simply dried, they shrivel beyond easy recognition. So do the eggs, larvae, and pupae of most insects, and the adults of such diverse kinds as termites, silver-fish, aphids, and many others.

Soft-bodied arthropods can be dehydrated artificially, with comparatively little distortion, by methods outlined later in this paper. The processes are, however, uncertain, laborious, and exacting. Since small but important characters are sometimes obliterated by desiccation, only those specimens which are required for exhibition, rather than for study, should ordinarily be mounted dry.

LIQUID PRESERVATION

Study specimens, the majority of those taken by any serious collector, are usually killed and permanently preserved in ethyl alcohol somewhat diluted with water, as described in Leaflet Number Three. If you are interested in soft-bodied insects and spiders of all sizes, you will find it convenient to have at hand, in labeled bottles, stock solutions containing ethyl alcohol in various proportions. Assuming the available alcohol to be "completely denatured," and of the best quality, it will contain about 95% of ethyl alcohol and 5% of methyl alcohol and gasoline. The relative volumes of denatured alcohol and water in the most useful mixtures will then be these:

Approximate concentration of ethyl alcohol	Parts by volume denatured alcohol	Parts by volume water	Use
50%	10	9	For killing very delicate specimens.
60%	12	7	For "stepping-up" delicate specimens. Rarely needed.
70%	14	5	For preserving delicate specimens and killing tougher ones.
80%	16	3	For preserving specimens of average or large size.

If you have, or can borrow, a measuring graduate of 100 cubic centimeters capacity, you can easily prepare alcohol of any given concentration from that of any known greater concentration in this manner. Fill the graduate with alcohol to the mark corresponding to the desired percentage of the mixture. Then add water until the total volume reaches the mark corresponding to the known percentage of the original alcohol. Thus, to make a 70% mixture from a 95% solution, fill the graduate with alcohol to the 70c.c. mark and add 25c.c. of water, making 95c.c. in all. The proportions listed in the table above were derived in this way, and although only approximate, are accurate enough for practical purposes. If the water in your district is conspicuously "hard," you might use distilled water in mixing your reagents, though "soft" tap-water is usually all right.

Alcohol of full strength, 95% can be used as a preservative for the larger species, but it will probably make them so rigid that they must be soaked in water before they can be manipulated for examination.

If your collection must go for long periods unattended, so that it is in danger of drying out, add to the stock preservative solutions about two precent of

glycerine, or two tablespoonfuls to a quart. Glycerine will keep the specimens pliant long after the alcohol has disappeared. Do not use it, however, if you handle your collection frequently. You cannot avoid spilling a little of the liquid as you work; and when the alcohol evaporates, the residue of glycerine on tools and hands is unpleasantly sticky.

Whether killed with alcohol in the field or with hot water in the laboratory, fresh specimens are placed, at first, in a solution which is too weak to preserve them permanently. It will, however, begin the dehydration without distortion. When they are transferred to a stronger preservative a day or two later, the specimens will not shrink as much as they would have done if dropped directly into the concentrated solution. The more gradual this "stepping-up," the less the shape of the specimens will be affected. Some biological preparations require a long series of baths differing in concentration by as little as five percent, but for most insects and spiders, one step is probably enough. The smallest and juiciest species may be the better for two. The concentrations recommended for these purposes are listed in the dilution table above. If a bottle contains a large number of bulky specimens, change the final preserving fluid at least once, after an interval of several days, before storing the bottle away indefinitely. The body fluids of the animals may dilute the first alcohol bath below the 70% minimum effective for long term preservation. As often thereafter as the alcohol becomes discolored, replace it with a fresh supply.

When collecting in a remote area, far removed from your base of operation, and with a limited supply of alcohol, changing the fluid within a few days will be impractical. In this case, it is better to collect into straight 95% alcohol, risking the distortion, than to chance losing the specimens through dilution of the preservative by body fluids. Replace the killing fluid with fresh alcohol of appropriate concentration as soon as opportunity arises.

It is likely that each collecting bottle filled in the field will contain specimens of several species, and possibly of different dates or localities. Before these can be incorporated into a scientific collection, they must be sorted and separated, so that one bottle contains creatures of one species, date, and locality only. You can do this most conveniently while changing the alcohol for the last time. When sorting a collection, be sure that every bottle, whether containing a single specimen or a dozen, also contains a label giving the locality and date of capture and the name of the collector. Field notes relative to the animals may also be included, as well as the name of the species, if you know it. Write these labels with black waterproof India Ink, on hard but unglazed paper of good quality. Make them of such size and shape that they fit into the bottles easily, and can be read conveniently through the glass.

To assure the workmanlike neatness of your collection, use storage bottles of one shape and a few standard sizes. Two common kinds are shown in Fig. 1. The homeopathic vial, "A", is preferable for a large or little-worked collection, because the constricted neck grips the stopper tightly, retarding evaporation of the alcohol. The shell vial, "B", must be refilled more often, but its use in small much-handled collections is justified by the ease with which the specimens may be extracted from it. Your local

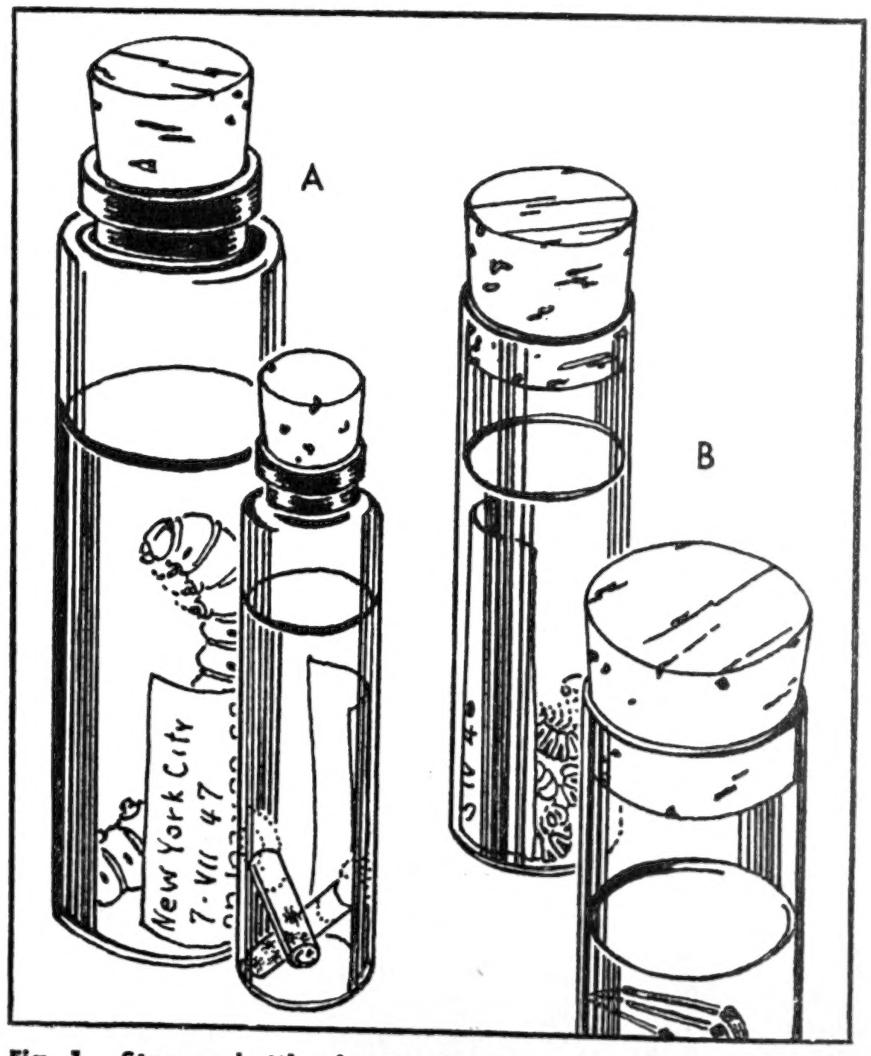

Fig. 1. Storage bottles for preserved specimens. A., homeopathic vials. Note tubes for minute specimens in the smaller. B., shell vials.

druggist probably carries vials of these or other suitable shapes. If not, you can buy them in quantity from any scientific supply house. Four drams is the most generally useful size, while eight drams will accommodate the largest species which you are apt to catch in this part of the world (New York). Vials smaller than two drams dry out so quickly that they are a nuisance. If you are afraid of overlooking extremely minute specimens in a large container, store them in tiny tubes stoppered with cotton, putting these tubes into the vials of alcohol. Such tubes are shown in the smaller vial at "A" in Fig. 1.

Rather than screw caps, bottles used for alcoholic specimens should have corks of the very finest quality. If the only available corks are coarse or faulty, use rubber stoppers. Store the bottles in an upright

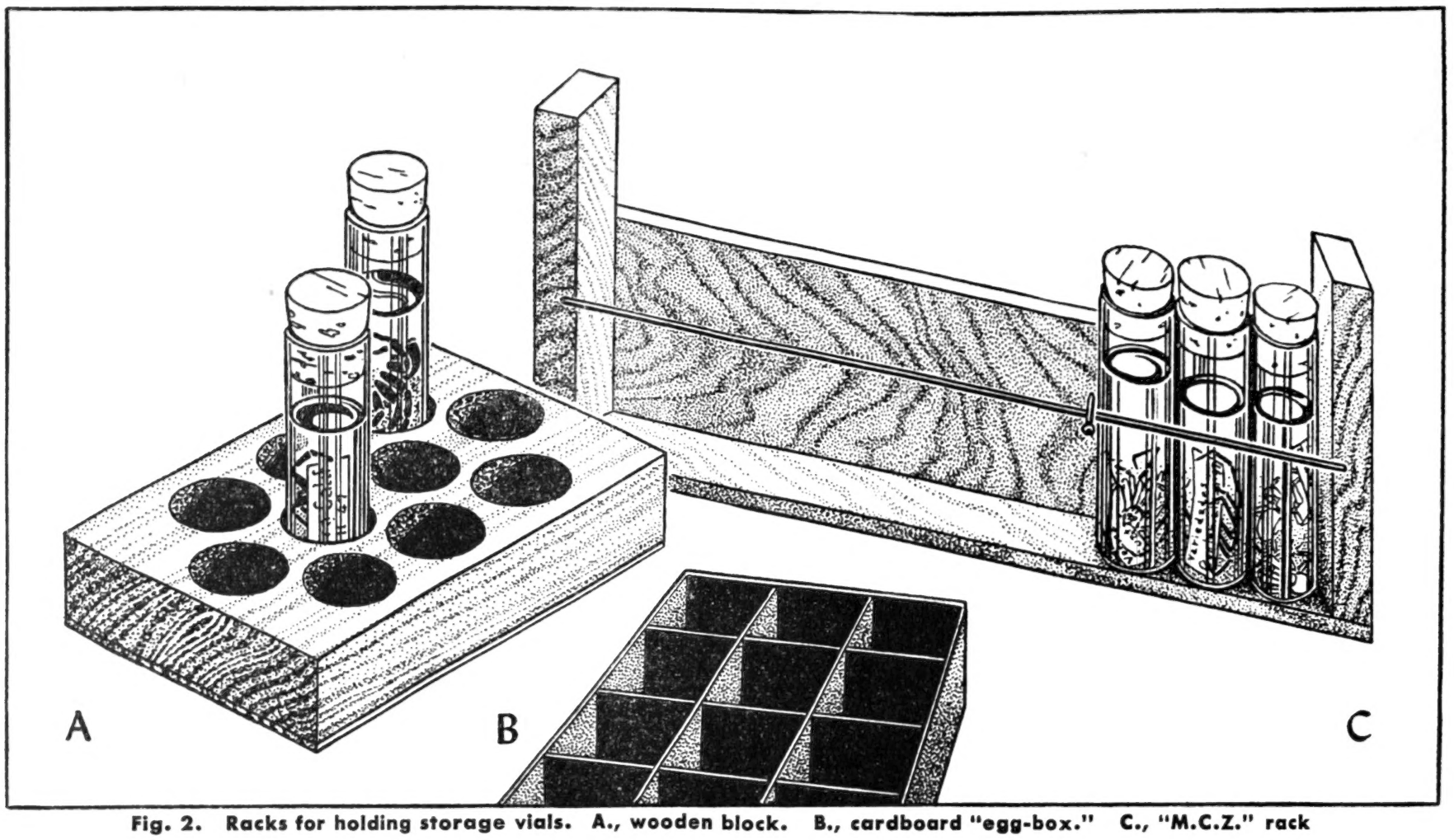

Fig. 2. Racks for holding storage vials. A., wooden block. B., cardboard "egg-box." C., "M.C.Z." rack

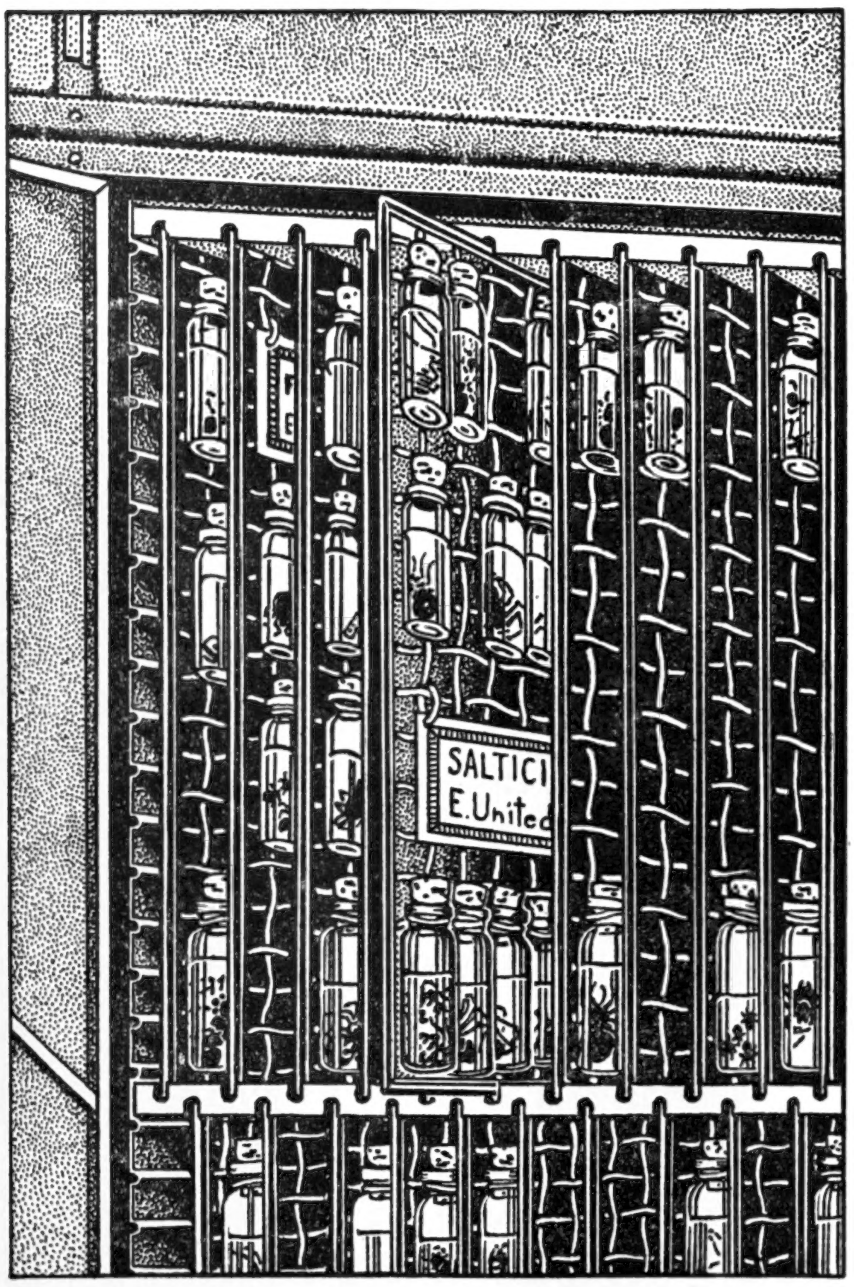

Fig. 3. American Museum method of storing an alcoholic collection.

position so that the liquid does not touch the stoppers constantly. Prolonged contact with alcohol hastens the deterioration of rubber and shrinks cork, allowing the liquid to escape.

Tall slender bottles readily become upset or disarranged. Avoid this by keeping them in racks or trays. Fig. 2. shows several kinds of racks which you can easily make for yourself. "A" is a piece of one-inch plank in which have been bored neat rows of holes a little larger in diameter than the largest bottle which they may be expected to receive. These holes are closed at the bottom by a thin piece of wood or a sheet of cardboard, fastened to the block with glue and tacks. "B" is a box divided into bottle-size compartments by cardboard partitions like those of an egg crate. The racks, like the bottles, should be of one design throughout the collection. In size they may vary, but they should all be made in multiples of a basic set of measurements, so that they will fit together without waste of space. Arrange the bottles in the trays, and the trays upon shelves, in some orderly manner; perhaps in the sequence followed by the author of the book which you use to identify the animals. You will then be able to find, without trouble, any species which is represented in your collection. By preference, the shelves should be enclosed in a closet or cabinet. It is no small chore to dust a collection of bottled arthropods.

"C" in Fig. 2. is an "M.C.Z." rack, used by the Museum of Comparative Zoology at Harvard. One side of the narrow box-like tray consists of a heavy

wire, exposing the contents of the vials to view. The ends of the tray are a little higher than the tops of the corked bottles, to protect the corks from being accidentally dislodged. They also serve as handles for lifting the tray out of the shallow drawer in which it is stored. Some "M.C.Z." racks are made double, with a wooden partition in the middle and wires on either side. These contain two rows of vials. There are no partitions between the individual bottles in the row. When the rack is not full, the bottles are held in position by a large pin driven into the wooden side horizontally, just above the wire, as the picture indicates.

Fig. 3. illustrates another and even more efficient way to store a large collection of alcoholic specimens.

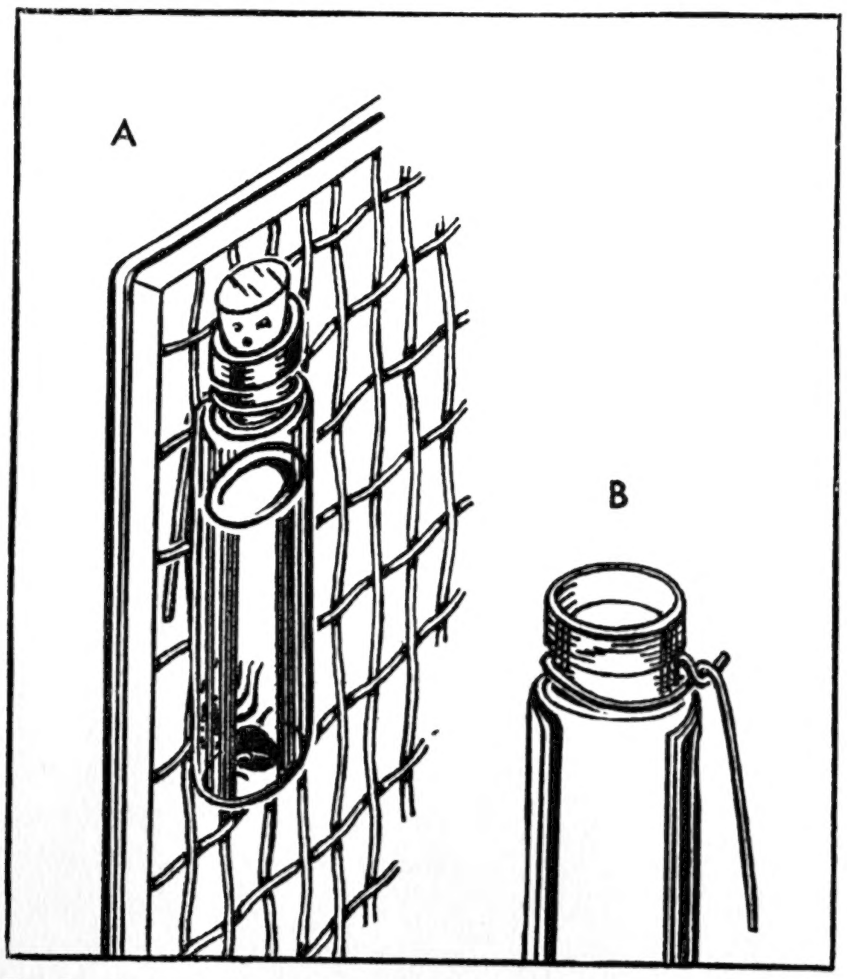

Fig. 4. A., corner of A.M.N.H. rack, showing metal binding. B., top of vial, showing how wire is twisted around the neck to form a hook.

It was invented and first used at the American Museum of Natural History. The creatures are kept in homeopathic vials. Around the neck of each vial is a collar of soft iron or copper wire prolonged into a hook, as you may see at "B" in Fig. 4. By means of these hooks, the vials are suspended in rows from vertical racks made of half-inch mesh galvanized wire screen. The rectangular mesh panels are bound around the edges with thin sheet metal. See "A" in Fig. 4. They are made to slide in grooves cut in the upper and under surfaces of the heavy wooden shelves with which the storage cabinets are provided. Since the slots need be no more than an inch and a half apart, and each panel will carry several rows of bottles, the capacity of such a cabinet is very great.

A bottled collection which must lie idle for long may very wisely be stored in pint canning jars as shown in Fig. 5. Remove the corks from the vials and replace them with cotton plugs. Cover the bottom of a pint canning jar with a thin cushion of cotton. Pack the vials into the jar, upright and as snugly as possible. Put on the rubber ring, fill the jar with alcohol, and clamp down the lid. Though years pass before you look at them again, specimens so stored will be moist and perfect when wanted.

Animals which have been preserved in alcohol should never be allowed to dry out. Unless your collection is "canned," inspect it at least once in six months, filling the bottles and replacing defective corks. When you want to study a specimen, remove it from its bottle with forceps and put it into a watch-glass or other small dish full of alcohol. Very little specimens can be transferred more easily by sucking them out of the container with a medicine dropper, fluid and all. A specimen which has accidentally dried can be relaxed by soaking in warm water, but it will never entirely regain its former appearance. A drop of some detergent or "wetting agent" such as Aerosol OT, applied directly to the specimen before covering it with water, will hasten the softening and frequently restores plumpness.

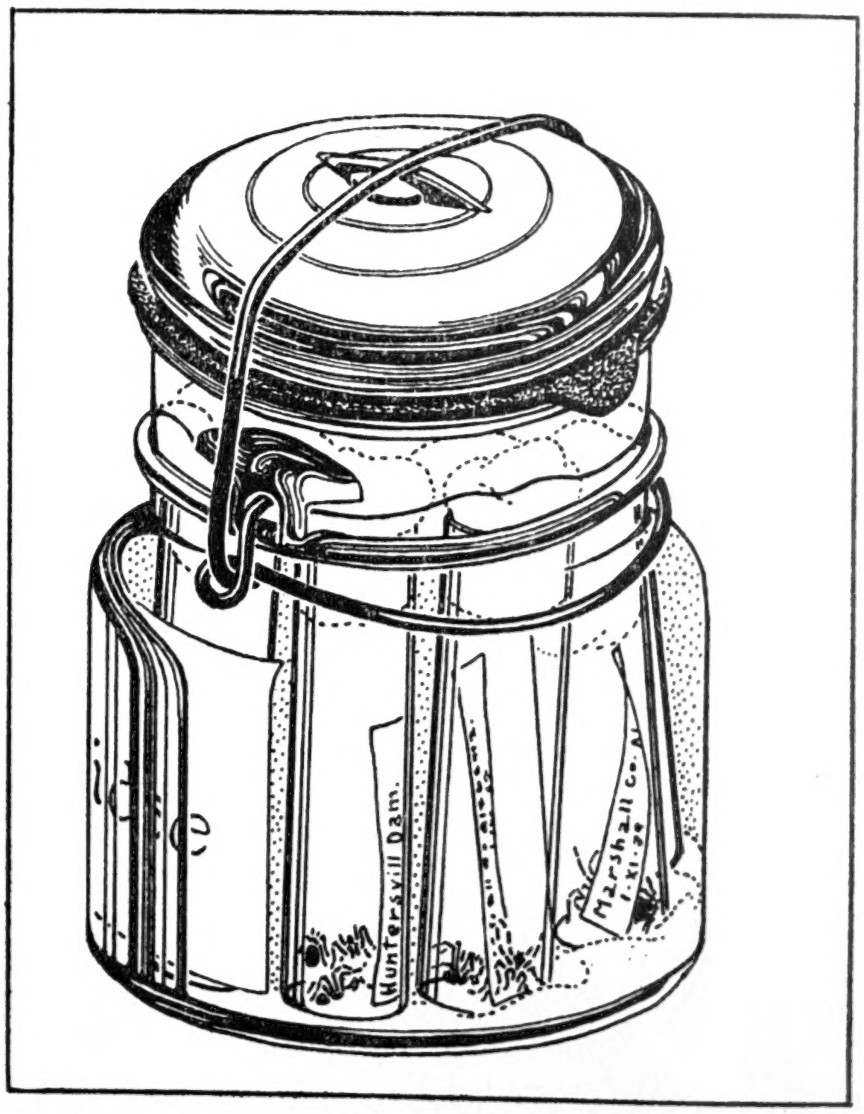

Fig. 5. Bottled arthropods stored in a pint canning jar, to prevent drying-out.

ARTIFICIAL DEHYDRATION

Although liquid preservation is more satisfactory for scientific purposes, it is sometimes desirable to mount spiders or soft-bodied insects dry. With the exception of Lepidopterists, students of insect development who like to keep together all the stages of a life cycle, hard and soft, are more likely to put the hard ones into liquid than to dry the soft ones. Show-specimens, however, should present as life-like an appearance as possible, and need not stand close expert scrutiny. These you will be justified in drying out by strategy.

Unhappily, no one stratagem will suffice for all occasions, and some specimens fail to respond properly to any process yet devised. Nevertheless, there are a few basic schemes by which more or less satisfactory dry preparations can be made from most soft-bodied arthropods. Once you have grasped the principles, you should be able to improve upon the skeleton directions here given, and with the experience of practice, to produce quite creditable results.

Specimens which have been preserved in liquid for a long time are sure to come out almost colorless when dried. Even the brightest of fresh specimens will dry slightly faded. If constantly exposed to a strong light, the remaining color soon bleaches to a neutral tan, only a ghost of the original pattern surviving. To preserve any semblance of their appearance during life, exhibition specimens will have to be painted. This should be done while you can still remember what they looked like, or better yet, while living specimens are available to serve as models. Ordinary oil paint is all right, but the transparent oil colors made for tinting photographs are particularly good for this purpose. They are so clear that a pattern of light and dark areas shows through, and can, therefore, be followed easily. Thin the paint with xylol for quicker drying, and put it on lightly with a small water-color brush. The more skill and patience you can bring to this operation, the more convincing the specimen will look.

DRY MOUNTING BY INFLATION

"Blowing" or "inflation" is the traditional and most widely practiced method for dry-mounting caterpillars. It can also be applied to grubs and to other creatures of suitable size and construction. The process consists of eviscerating the specimen, filling it with air like a balloon, and blowing continuously while it is drying in a small oven. For about six dollars you can buy from a scientific supply house a complete set of equipment for inflating caterpillars, together with instructions for its use. It is, however, perfectly practical and much cheaper to make your own "blowing" outfit from odds and ends, most of which you will already have around the house.

The principal item is the oven. A tin can of moderate size is readily converted to this use, as shown in Fig. 6. Open the can as neatly as possible, leaving the top attached to the body for about two inches. Empty and wash the can, removing the paper label. With tin shears, cut in the lid a wedge-shaped hole large enough to admit a big caterpillar. Then bend the lid back to its original position in the mouth of the can. At the other end, just under the opening, puncture the can with a beer-opener, to make a vent or flue.

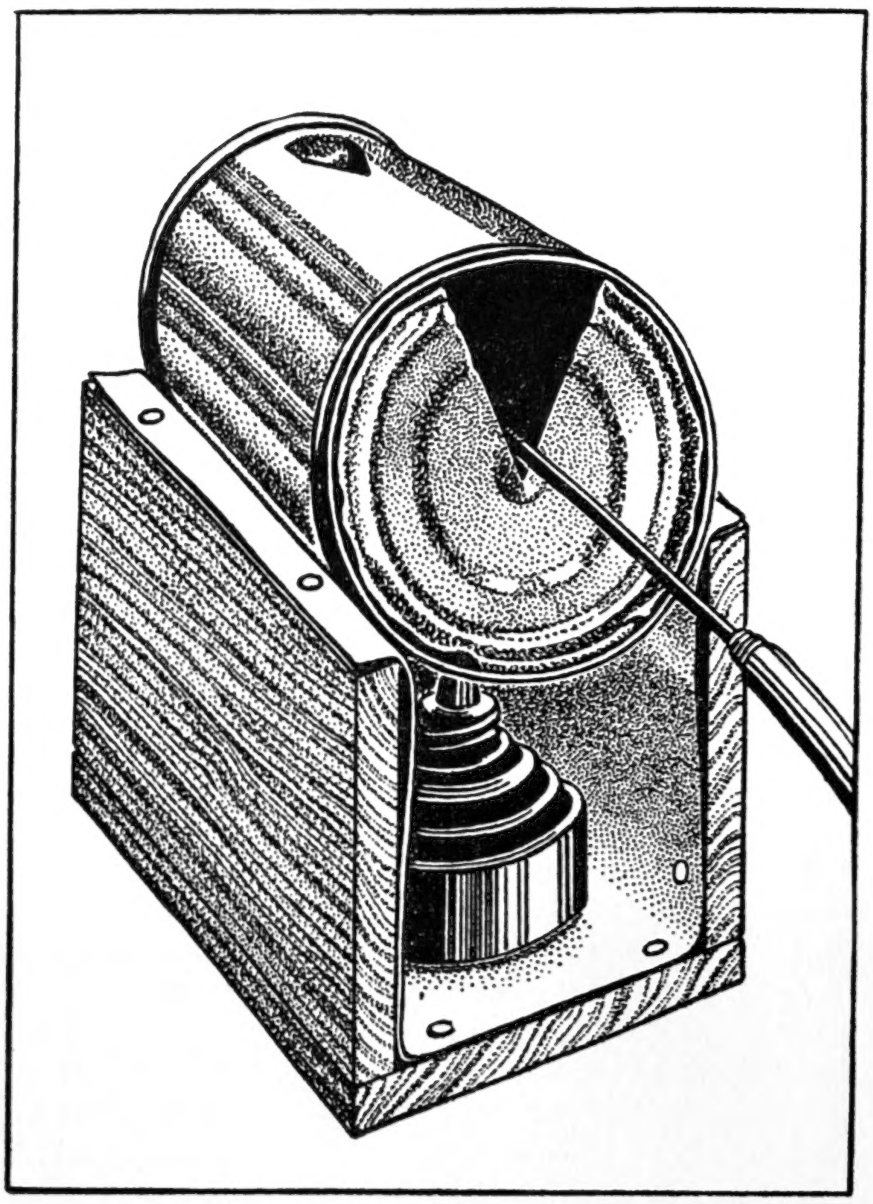

Fig. 6. Home made oven for drying inflated caterpillars.

The stand used to support the oven in the picture is made of scraps of wood, nailed together and lined with metal from another can. The source of heat is a spirit lamp, such as any druggist can supply. With a little ingenuity, you can adapt your oven to a gas ring or electric hot plate; but if you do, be sure to keep the fire turned low. Specimen, equipment, and operator alike may be damaged by excessive heat.

To "blow" an insect, air is forced into the skin through a fine tube or canula. In a purchased outfit, this tube will be of metal or of glass, tapering at one end and equipped with an adjustable clamp to hold

the specimen. See Fig. 7., "C". You can make one just as good from a fine-drawn medicine dropper and a pair of bobby pins. The pins, which are made of spring steel convenient in size, have only to be cut or broken at the bow. The hooked portions are bound to the dropper with heavy thread, as shown in Fig. 7., "B". The early entomologists never bothered with such pretty gadgets; they used straws, not the soda fountain variety, but real old-fashioned grass stems. You could do worse than follow their example, since the straw, becoming a permanent part of the specimen, simplifies both the process of inflation and the subsequent mounting of the dry skin. You can find suitable straws in any field or vacant lot, or if need be, in the kitchen broom. Select them of various diameters, and as long as possible, inspecting each to be sure that it is free of obstruction. Keep an assortment at hand, together with a spool of fine thread or a box of needle-points with which to attach the specimens.

For best results, the flow of air through the specimen should be constant and steady all the while the skin is drying. This may be as much as half an hour. Even a glass blower might have trouble in maintaining a constant pressure for so long a time by lung-power, but the nearer you can come to it, the better. If you plan to "blow" many caterpillars, you would be well advised to buy a "constant pressure bulb" from a scientific supply house. It will cost two dollars, at the most. Made of rubber, and shown in Fig. 7, it is operated by squeezing the bulb furthest removed from the specimen. This pumps air into the second, or storage, bulb, whence it escapes in a steady stream regulated by a valve at the exit.

When inflating caterpillars by mouth, you will find it tiring and uncomfortably hot to keep your face constantly a bare straw's-length away from the stove. Eighteen inches of small rubber tubing attached to the straw will give you welcome freedom. If the straw is too small for the tubing, wrap the end with a strip of paper until it fits snugly. A glass mouthpiece made of a medicine dropper inserted at the other end of the hose is an added refinement. Incidentally, ask for pure natural rubber tubing when you order it. Regard all synthetic plastic compounds with suspicion. The flavor of the fumes given off by some of them, especially when hot, must be experienced to be believed!

In preparing a caterpillar for "blowing," you will need some absorbent paper, such as towelling, napkins or cleansing tissues; a pair of forceps; a pair of small scissors; and if the specimen is too large to cover with your finger tip, a roller, such as a pencil or a vial.

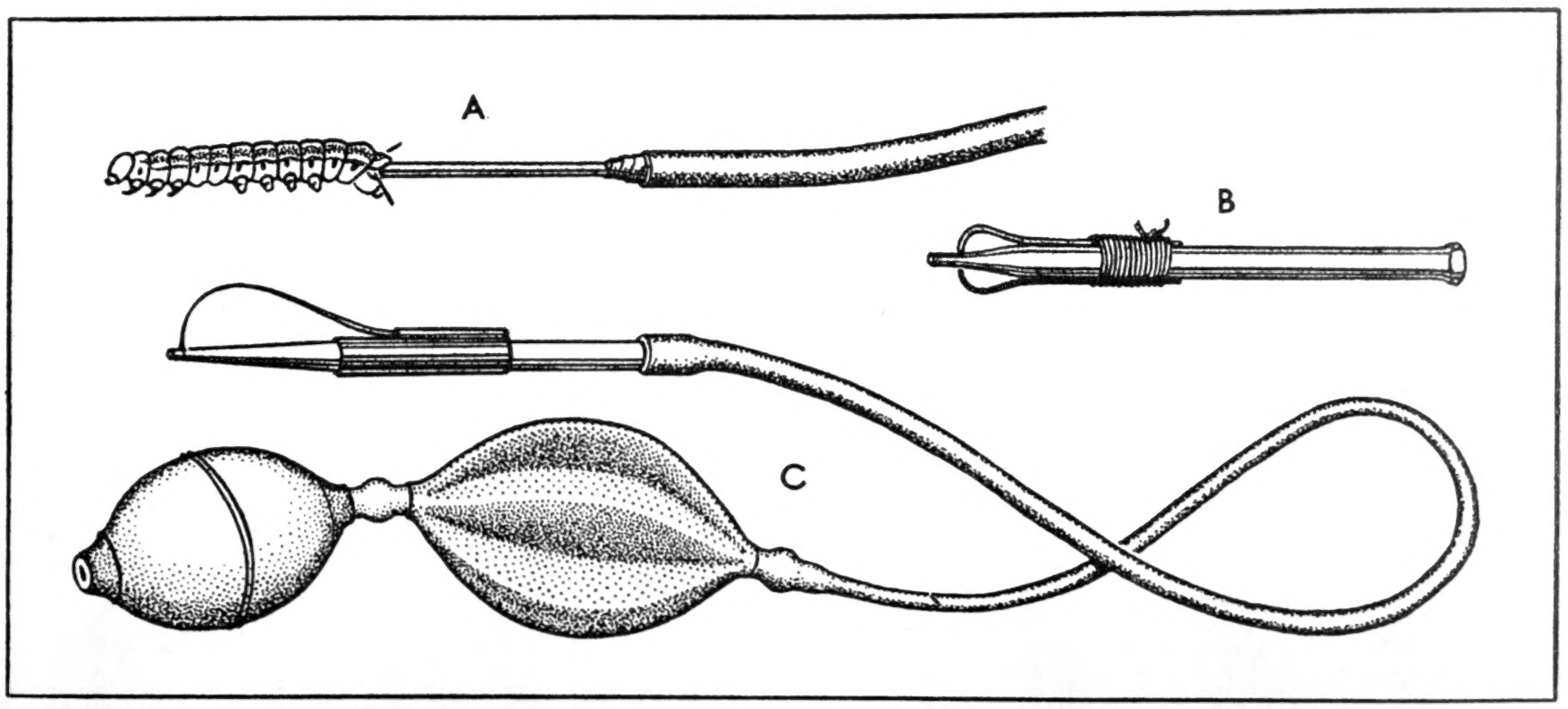

Fig. 7. Inflating apparatus for caterpillars. A., Specimen pinned to a natrual straw. B., Medicine-dropper canula with double boby-pin clamp. C., Commercially made outfit with constant-pressure bulb, and single clamp.

The animal selected for your first attempt at inflation should be a moderately large caterpillar, tough of hide and free of hairs and spines. It should also be of a common species, so that its loss, in case of failure, will not be too keenly felt. Delicate, small, and pilose specimens are more difficult to handle. These, together with the rare and precious, should be postponed until you have achieved a little skill.

If the specimen has not been preserved in liquid in the field, kill it with a poison bottle, or by dropping it into hot water. Lay it on a pad of absorbent paper. Then light the fire under the oven, so that it will be warm by the time you want it.

With the fingers of one hand, press the larva firmly against the paper, as though to flatten it. This will

cause the intestine to protrude at the anus, where it can be severed with the scissors. Working from head to tail, with a finger or the roller, squeeze out the body contents. The more fluid portions will be absorbed by the paper, and the whole operation is much quicker and less messy that you would expect.

When the skin is as empty as you can get it without bruising it, insert the air tube at the anal incision. If you use a straw, choose one to fit the opening. Run it into the specimen as far as the thorax so that it will act as a support to the limp hide. Fasten the specimen securely by means of a thread tied around it as close to the anus as possible, or by one or two needle points driven into the straw through the skin, as illustrated in Fig. 7., "A". A canula will be somewhat tapered. Insert it as far as necessary to make it fit closely, and

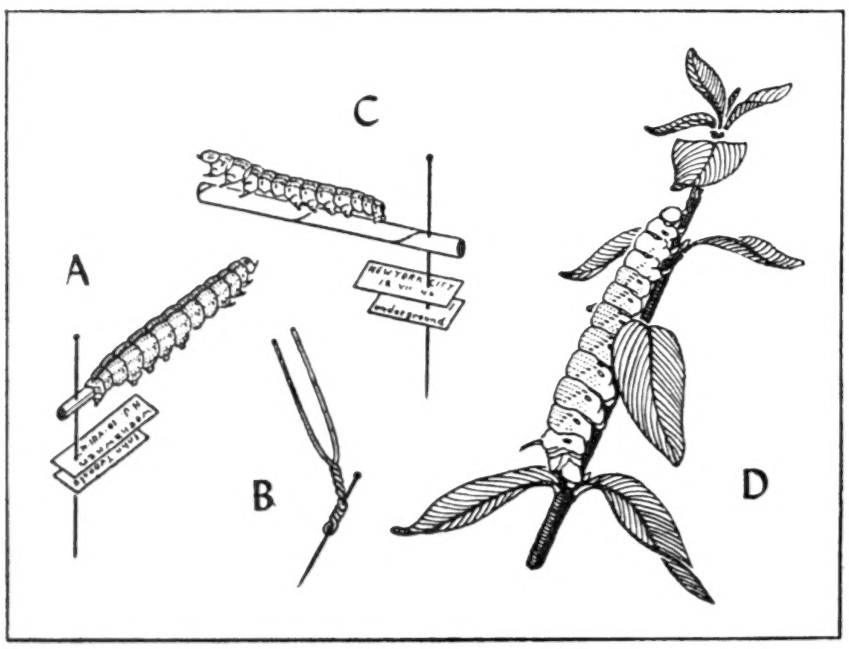

Fig. 8. Methods of mounting dried caterpillars. A, B, and C. Study-collection mountings. D. Exhibition mounting.

adjust the clamp to hold the specimen. Since this in no way supports the empty skin, a constant pressure bulb is almost necessary to prevent collapse whenever you stop blowing for a second. Of course, you can stand the oven on end and hang the specimen in it, head down; but this will call for a mechanical means of holding the tube in place, since the heat rising upward from the oven is too great to be endured for long by unprotected hands.

Inflate the larva by blowing gently on the other end of the tube, and insert it into the oven through the opening provided for the purpose. The air pressure should be maintained as steadily as possible, but it should not be great enough to distend the specimen further than it could expand in life. Rest the tube in the V of the opening as you blow. From time to time, remove the specimen to see how it is progressing. When done, it should be perfectly dry, hollow, and rigid, so that it can be held between the fingers without collapsing. Its color should be almost natural. The time required to achieve this desirable condition will vary with the size and density of the specimen, the temperature of the oven, and the humidity of the atmosphere. Do not leave the specimen in the oven longer than necessary, lest it burn.

If you have used a straw to inflate the creature, do not remove it, but cut it off about a quarter of an inch behind the specimen and pass an insect pin through the stub. Remove any needle points which you may have used, but leave a thread, after clipping off the ends. If you have used a canula, remove the clip and loosen the insect with a needle. Withdraw the tube with a cautious, twisting motion.

Several methods of mounting "blown" caterpillars are shown in Fig. 8, "A" being the pinned straw mounting already described. "B" is a device for holding a strawless specimen. It consists of a piece of fine silk-insulated copper wire about twice as long as the insect, bent double, with the part nearest the bend wrapped several times around an insect pin. The free ends of the wires are twisted together for a short distance, close to the pin, before being spread apart to produce a long Y a little wider than the insect. To use, cover the ends of the wires with glue, shellac or plastic cement; pinch the wires together at the base of the fork; and insert both ends into the opening left by the canula. The further they project into the specimen, the more support they will give it. When the pressure is released, the wires will spread apart, and be stuck to the sides of the insect by the adhesive with which they are covered. Wire which is too stiff and strong may burst the specimen.

At "C" is shown a caterpillar mounted by gluing its feet to a straw, natural or soda-fountain variety, which is impaled upon a pin. Note that any caterpillar mounted as part of a scientific collection is provided with pin labels giving the locality and date of capture, the captor's name, food plant when known and other pertinent details, before being pinned into the cork-lined box beside the adult of the same species. Pin labels and storage boxes are discussed in Leaflet Four.

"D" shows how method "C" may be adapted for an exhibition specimen, a real twig with artifical leaves replacing the less artistic straw. If the plant copied be appropriate to the species of caterpillar, the mounting will be instructive as well as attractive. It would be adviseable to paint the specimen, since exposure to light will soon bleach it. To minimize the rigidity of the inflated larva, mount it on a spot where its linear posture will seem natural.

If you are interested in exhibition mounting, you will learn a great deal about the making of artificial foliage, fruits and flowers from the Museum's Science Guide No. 82, "Building the Museum Group." This you may have for the sum of eighteen cents, including postage, by writing to the Division of Popular Publi-

cations, The American Museum of Natural History, Central Park West at 79th Street, New York City.

MICROSCOPE SLIDES

Of the million or more known species of arthropods, a great many are too small to be appreciated by the naked human eye. Of the hypothetical ten million yet to be described, the majority are probably minute. Adequate study of such creatures is impossible without the aid of some sort of magnifier, and if you are an enthusiastic student, you will want the best that you can find. The best is not necessarily the strongest. With increasing magnification, the size of the field and the depth of focus decrease, so that less of the specimen is visable at a time. For most insects and spiders, a four- or six-power hand-lens is more satisfactory than one with a magnification of twenty diameters. Take a few specimens with you to the opticians's, and try a variety of lenses critically before making your choice.

For laboratory work, a low-power binocular "dissecting" microscope is better than any hand-lens; but since it costs over a hundred dollars, few amateurs can hope to possess their own. Most high-school and college biology laboratories do have such instruments. Perhaps you can arrange to use one of these, when the class is not in session. Have someone who thoroughly understands it demonstrate its operation. You will be amply repaid for any trouble you may take in learning to use the microscope properly.

Specimens which are so small that they must be examined through lenses are likely to be correspondingly fragile. They should be handled as seldom as possible. Hard-bodied ones may be mounted dry, on cardboard "points," as described in Leaflet Four. Soft-bodied ones may be preserved in liquid, as discussed above. Either may be mounted upon slides.

The standard microscope slide is a slip of clear glass, one inch wide by three inches long. The specimen, suspended in a transparent medium, is sandwiched in between this slide and a smaller and much thinner cover-glass. So mounted, it is relatively safe from damage, easy to handle, and convenient to store. There is, however, this serious disadvantage; the position of the specimen is fixed, and one, or at most, two sides only are exposed to view. For this reason, it is desirable, when practical, to mount several identical animals in different positions upon the same slide, so that what is concealed in one may be displayed in another. The distortion apparent in some slide-mounted specimens is largely the result of faulty preparation and can be avoided.

Slide making for the medical sciences is a fine art about which many scholarly volumes have been written. Entomological slide making is, fortunately, easier. To an histologist or a bacteriologist, the smallest whole insect or spider is a gross object which can be treated adequately by processes simple to the point of crudeness. This does not mean that you can afford to be careless. It does mean that, working with a minimum of equipment and with such materials as are readily available and relatively harmless, you can expect to produce a satisfactory product most of the time.

The method of slide preparation here described, though not quite the simplest possible, is yet frankly a beginner's method. It requires no expensive tools, and no chemicals which are extravagant, explosive, deadly in small quantities, or proscribed by law. Nevertheless, it is a workable method of mounting most kinds of small insects and spiders, and a sound foundation upon which to build more elaborate procedures.

Many of the necessary materials you have already; a large drugstore may be able to provide the rest. If not, you can order them from a scientific supply house. Here is a list of the things that you will need.

1. "Completely denatured" alcohol of graduated concentrations, including full strength, as described above under "Liquid Preservation."

2. Xylol (xylene). Although a liquid, this is sold by the pound. One pound is about a pint and a half, and should last a long time.

3. Carbol-ylene. Four ounces will be plenty. This is a saturated solution of phenol (carbolic acid) in xylene. Phenol is very corrosive, and can "burn" you badly. If you spill any of this mixture on your skin, wash it off at once with strong alcohol.

4. Canada balsam, the transparent medium in which the specimens are to be suspended. A resin soluble in xylene, balsam is usually sold as a viscous liquid, packed in a bottle or a tube. It is almost colorless when new, but turns yellow with age.

5. "Duco" or other clear plastic cement.

6. Containers for the specimens during processing. The number and kind will depend upon the scale and frequency of your operations. If you wish to make a few slides only, at long intervals, you may leave each lot of specimens in its own labeled vial throughout the process, changing the fluids with a medicine dropper. If, on the other hand, you expect to be working steadily for a season, you may prefer to keep each liquid in a marked and covered dish, and to transfer the specimens from one to another. In this case you will need:

7. Some gelatine capsules, such as druggists use for powdered medicines. You can buy an assortment of sizes at any pharmacy. When each end is punctured several times with a strong needle, such a capsule becomes a miniature strainer in which one or several specimens can travel safely through a whole series of baths. To prevent confusion, mark both the capsule and the data label of the insect which it contains with

a letter or number by which the two may be identified. The symbol may be scratched on the capsule with a pin, or written with India ink.

8. Slides. These come by the dozen or half gross. The price per slide falls as the quantity rises.

9. Cover glasses, available in several sizes, and in two shapes, round and rectangular. They are sold by the ounce. Half an ounce will be plenty to begin with.

10. Some supports to hold up the cover glasses and prevent the specimens from being squashed as the balsam dries and shrinks. Supply houses offer, for this purpose, cover-glass size plastic rings of various thicknesses called "cells." These are tidy to use, and look well, but they retard the drying of the balsam by excluding the air. It is just as practical, and cheaper, to support your covers with fragments of broken cover-glass, if the specimens are thin, and with bits of transparent plastic sheeting of appropriate thickness, if they are bulky. These stilts will scarcely show in the finished slide, and since they do not entirely surround the balsam, they permit it to dry more rapidly.

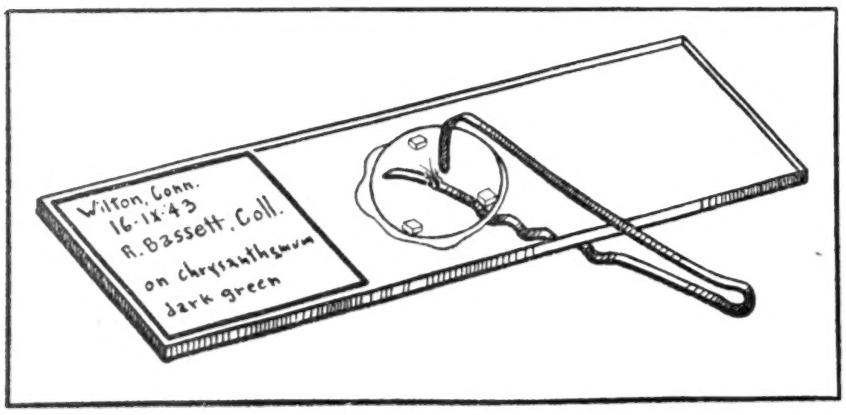

Fig. 9. Newly assembled microscope slide. Note label, cover-glass stilts, and bobby pin clamp.

11. Tools for handling the specimens, including fine forceps, mounted needles and medicine dropper.

12. Absorbent paper, such as blotters, towelling, or facial tissues.

13. Bobby-pins, bent into clamps for holding cover glasses as shown in Fig. 9. If the steel snaps when bent, grasp the bow of the pin with pliers and heat the ends red hot over a gas flame. Allowthe pin to cool slowly, without quenching. This will remove the temper, so that you can bend the metal easily.

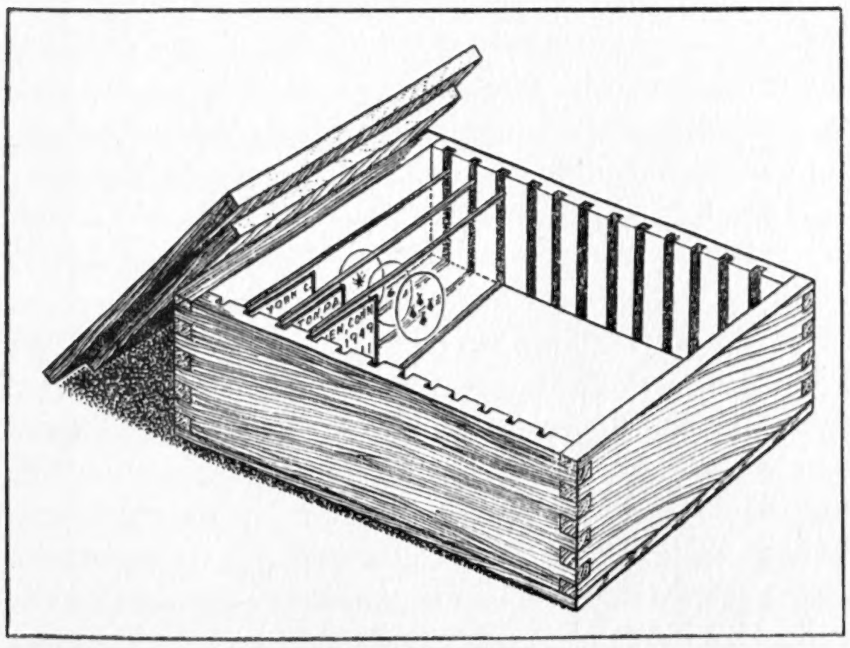

Fig. 10. A small storage box for microscope slides.

14. Gummed paper labels a little less than an inch square, fine pen, and India ink, for marking the slides.

15. Slide boxes in which to store the finished product. One of these is shown in Fig. 10.

16. A jig or pattern by which to center the specimens on the slides. Although not strictly necessary this is a useful gadget which will save you more time than its construction will require. Fig. 11 is a picture of a jig. To make it, you need two rectangular pieces of cardboard, about four inches by two. Lay a glass slide on the lower right-hand corner of one of the pieces, matching the edges, and draw a line around it. Cut out the corner of the cardboard, following the outline carefully. Glue the two pieces of card together, matching the three remaining corners, and put them to press under a heavy book until dry. You will now have a thin block of cardboard with a slide-size depression in one corner. With a ruler, located the mid point of this depression. Mark it. With this point as center, draw a circle or rectangle the size of a cover glass. This locates the area which the cover-glass will occupy on the finished slide.

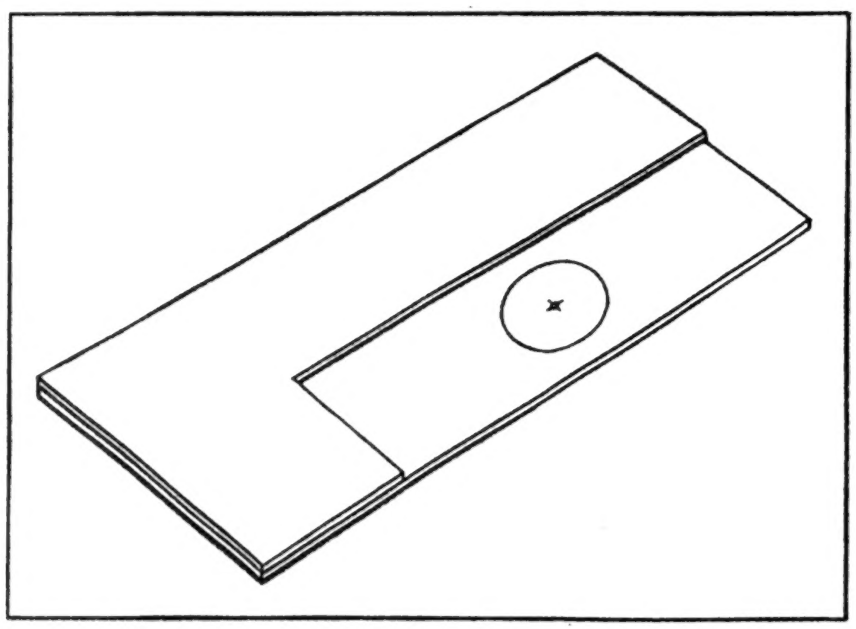

Fig. 11. A jig for centering specimens on microscope slides.

A biological specimen imbedded for scientific study must be not only covered but completely filled with resin to make it clearly visible. A trace of water in the specimen will fog the balsam. An air bubble inside the animal deflects the light and obscures the image. To replace the natural liquids and gasses of the body with a transparent, and ultimately solid, substance is the purpose of slide preparation processes.

The body fluids of animals are largely water. Balsam is soluble only in oils. The incompatability of

oil and water is proverbial. Fortunately, both water and xylol, the highly volatile oil used to dissolve the balsam, are miscible with alcohol. Thus alcohol can be used as an intermediate step between the two. In preparing a specimen for slide-mounting, the water in the tissues is wholly replaced by alcohol. During the process any air which may be trapped in the body cavities is, incidentally, dissolved. Then the alcohol is replaced by xylol and the xylol by a solution of balsam in xylol. When the oil evaporates, the solid resin remains, filling and covering the specimen completely and permanently.

In taking a specimen through the slide-mounting processes, the sequence of operations will be as follows:

1. Catch your specimen and kill it by dropping into hot water. This extends the appendages. Specimens killed in alcohol, although otherwise suitable, are likely to be difficult to arrange on a slide because the contracted position in which they are fixed. Prick the abdomen with a fine needle to admit fluids more readily.

2. Dehydrate the specimen by "stepping up" through a graduated series of alcohols. For most eggs and adult insects or spiders, however small, 50%, 70%, and 95% or full strength will be enough. For extremely minute or delicate immature forms, baths of 60% and 80% may be included. Leave the specimen in each bath for about an hour. Change the liquid on the specimen or the specimen in the liquid, as you prefer; but in either case, drain the creature thoroughly at each change. A bit of absorbent paper grasped with forceps is convenient for mopping up the last drop in the bottom of a vial. If you are using a capsule, press one end of it against a blotter. It will drain at once. When lowering a capsule into a bath, hold it vertically, so that the air may escape at the top as the liquid rises from the bottom.

One bath can be employed to process many specimens, if you are careful to avoid unnecessary dilution. Once used, however, the fluid should never be returned

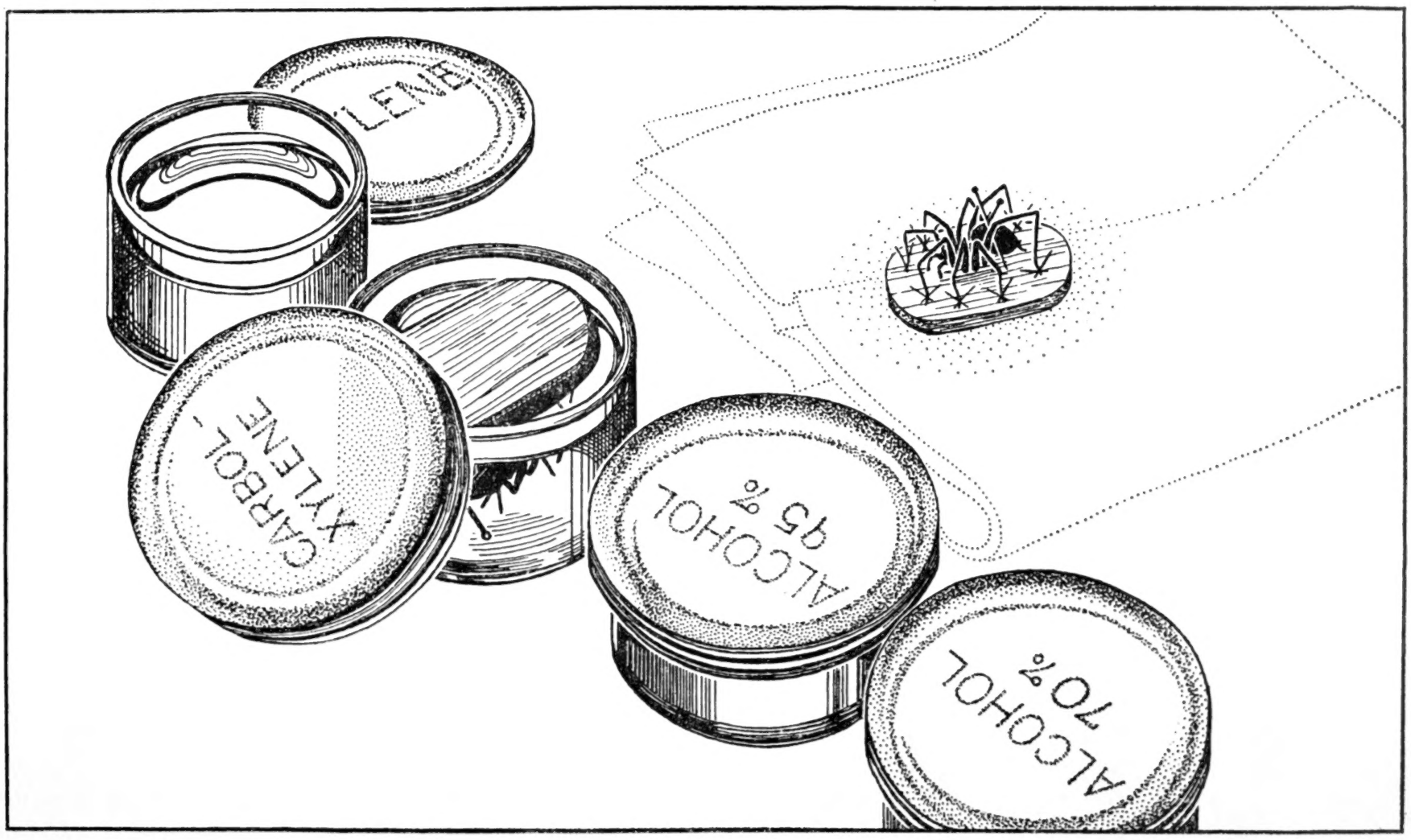

Fig. 12. Series of dehydrating baths for the preparation of spiders and soft bodied insects. The specimen, pinned to a chip of balsa wood, floats up-side-down in the dish.

to the stock bottle. Keep it in a special bottle marked "used." The 95% alcohol is the most critical step of the de-hydration, and should be renewed frequently. Save the old alcohol to feed the spirit lamp for the caterpillar oven.

3. From 95% alcohol, transfer the specimen to carbol-xylene. This serves two purposes. The phenol, which has a great affinity for water, will remove the last traces of moisture from the specimen. The xylol replaces the alcohol and dissolves the natural fats and oils of the body, making it transparent. This is called "clearing." If clearing fails to occurr after a few hours, you may reasonably suppose that the carbolic acid is exhausted, and the solution should be changed. Carbol xylene turns brown with prolonged

exposure to light, but this does not seem to impair its efficiency. It is rather viscous, and if you are using a capsule, you may have to open it to let this liquid in.

4. When the specimen is perfectly clear, rinse it in two baths of xylol to remove the phenol. Allow for each the same length of time as was required for the clearing.

5. Clean and polish a slide and cover glass. Use soap and water. Handle the polished glass by the edges only, to avoid finger prints. Beware of dust and lint. Select the cell, glass fragments, or bits of plastic sheeting to support the cover. Be sure that the tools, balsam, and "duco" cement are ready at hand.

6. With pen and india ink, copy the data relative to the specimen onto one of the gummed paper labels, and stick it to the extreme left-hand end of the slide. If there is more information than one label will hold, put another at the right-hand end.

7. Lay the slide in the jig. Place the cover glass supports in position, in the corners of a rectangle or evenly spaced around a circle, just inside the outline marked on the jig. Fasten each with a little clear cement. Place a large drop of balsam in the middle of the slide and spread it with a needle almost to the limits of the outline all around. Allow it to stand for several minutes to become tacky. Remove your specimen from the xylol and place it over the dot at the center of the jig. Spread out the legs and other appendages by the aid of needles. The tacky balsam will help to hold them in place. If several specimens are to be mounted on the same slide, arrange them in some orderly manner. Then flood each with a small drop of balsam, guiding the flow with a needle so that the air is forced out ahead of the liquid, and no bubbles are formed. Only experience can teach you just how much balsam you will need. Try not to spill or smear it on the glass where not wanted. It is a great nuisance to remove. If you get it on your clothes, you can wash it out with xylol or turpentine.

Specimens sometimes come loose and float out of position, even after the cover glass is on. If they are sufficiently tough and rigid, you can prevent this by gluing them to the slide. Instead of the first layer of thick balsam, put a small drop of thin plastic cement on the glass. Dry the surface of the specimen by blotting or blowing. Place the animal upon the drop of cement, and arrange the appendages. Then flood with balsam.

8. Pick up the cover-glass with forceps, and touch one edge of it to the slide at a point indicated by the outline on the jig. Lower the other edge slowly, so that as the glass touches the balsam, the air is driven out before it. Press the cover down gently, until it rests on the supports, and secure it in position by means of a bobbypin clamp. If there is not quite enough balsam under the cover to fill the space, apply a small drop to the slide at the cover's edge, at one side of the empty spot. Tip the slide to encourage the flow in the proper direction, so that no air is permitted to remain under the cover. If there is any extra balsam forced out around the edges of the cover, leave it there.

9. Check the centering of the cover glass by means of the jig, and set the slide away to dry. This will take a very long time, and during that while the slide should be kept clean, dry, and warm, with a good circulation of air if possible. It must remain in a horizontal position, and nothing should be allowed to touch its upper surface. The top shelf of a closet is a pretty good drying spot, if you do not forget what you have there and put a hat on top of the slides by accident.

Inspect the slide from time to time, as it dries. On the first day, you will find that the duco cement has turned milk white, but within a week it will have cleared again. As the xylol evaporates, the balsam shrinks, and empty pockets may form at the edges of the cover. If so, fill them with drops of liquid balsam. After about a month, it will be safe to take off the clamp. You may now examine the slide with the microscope, if you are very careful. However, it will be six months to a year before the balsam is dry enough to permit you to put the slide away in a slide box in a vertical position, or to examine it from the back without grave danger of dislodging the cover-glass. It is doubtful if very thick cell-mounts ever become completely solid. When the cover is so firmly cemented to the slide that you cannot shift it by a gentle shove, you may remove unsightly smears of balsam with a cloth moistened in xylol. There is no objection to leaving a neat ring of balsam around the cover. Indeed, it acts as a protection to the fragile edges of the glass.

It is possible, though risky, to hasten the drying of balsam by heating the slide carefully over a spirit lamp; but if you are ingenious as well as impatient, you can combine a metal box, an electric lamp and some bits of screen to produce a drying oven which will safely reduce the drying time to a few days.

10. Store your finished slides in a slide box, or boxes, arranged in some convenient order, so that you can always find the one you want without looking through the whole collection. The usual plan is to arrange them taxonomically, that is, according to their scientific classification, in the order given by a book of reference. You may find it easier or more interesting to arrange them ecologically, that is, according to the kind of place in which you found them, all the apple tree insects in one place, and all those that live on oak-trees in another, for instance.

Slide mounted specimens are usually viewed by transmitted rather than by reflected light. That is, the light shines through them from below. There are

insects so dark and dense that even when cleared in xylol they obstruct the light and are visible only as silhouettes. Such specimens are often improved by bleaching. Naturally colorless and very thin-skinned specimens may become invisible when cleared. These should be dyed. The stain used to color these specimens will not "take" unless they also have been treated briefly with a bleaching solution, so the very dark and the very light require the same kind of special treatment before the dehydration begins.

The bleach commonly used for this purpose in laboratories is a 10% aqueous solution of sodium hydroxide. Common household "Chlorox," which is a 5% aqueous solution of sodium hypochlorite, does about as well. Put a small quantity of the bleaching solution in a glass dish, and place the dish on a white paper so that can see the specimen easily. Transfer the specimen to this solution from the first alcohol bath. Watch it closely, through the microscope if possible. Great care is necessary to avoid over bleaching. When the appendages become pale and easily movable, it is time to stop. Further treatment will dissolve the membranes of the joints, and the insect will fall apart. Rinse the specimen in clear water to remove the bleach, then return to dilute alcohol and continue the preparation as with unbleached specimens. Bubbles of gas are sometimes formed inside of the body during bleaching. These will probably dissolve before the preparation is completed. If still present when the insect reaches the final bath, squeeze the abdomen gently to force the bubbles out through the needle hole.

There are many stains which may be used to tint colorless specimens. Basic fucsin is one of the more commonly used. It is a clear purple-red dye which you can buy, ready to use, from a scientific supply house. Apply it to specimens which have been bleached and run up to 95% alcohol. Put a little of the dye in a small container and allow the specimen to soak in it for a minute or two. Rinse in 95% alcohol, If the insect is not bright pink all over, dip it again. but do not permit it to become too dark. After dying and rinsing in alcohol, continue the preparation with carbol-xylene, xylene, and balsam, as has already been described.

After seeing what this method of slide preparation can do, you might like to try more complicated and professional ones. For expert advice, consult an article called "Mounting Aphids and Other Small Insects on Microscope Slides" by E. O. Essig, which was published in the January, 1948, issue of the "Pan-Pacific Entomologist. If you do not have access to a museum or university library which subscribes to this journal, you can obtain a copy for seventy-five cents by writing to the California Academy of Sciences, San Francisco 18. California.

DRY MOUNTING BY CHEMICAL DEHYDRATION

There are many large soft-bodied arthropods which cannot readily be inflated. Some of these can be dry mounted by replacing their natural moisture with a liquid which so hardens the tissues that they shrink but little when that liquid is removed. To minimize distortion, the replacement is accomplished by degrees, delicate and succulent specimens progressing more gradually than leathery ones. The simplest method is to remove the water with alcohol and the alcohol with xylene, exactly as though the animal were to be slide-mounted. Xylene evaporates rapidly, leaving the specimens dry, rigid, and usually unwrinkled, but somewhat smaller than in life. Although a pattern may persist, color will be almost wholly lost. Species which are transluscent when living come out perfectly opaque.

The reagents used to dehydrate large arthropods are those used in slide prepation: 70 and 95% alcohol, carbol-xylene, and xylol.

Additional equipment includes:

1. Stender dishes or small covered jars, 2 or 3 inches deep and big enough around to accommodate the outstretched legs of your largest specimen. If you are preparing one specimen only, one dish will do. Change the liquids. If you are going to mount a series, provide one dish for each bath, and transfer the specimens.

2. Insect or dressmaker's pins, and if possible, some needle points or "minuten nadeln."

3. A few pieces of cork or balsa wood about 1/8" thick and small enough to fit easily into the dishes.

4. Forceps, with which to handle the specimens and the small pins.

5. Oil colors, for restoring the pigmentation of the finished product.

Reagents, dishes, and other equipment, if not obtainable locally, should be ordered from a reliable scientific supply house.

As an example of the process, let us suppose that you wish to mount a large spider in a walking position. First, observe how it stands in life, the height of the body from the ground and the attitude of the legs. Then, kill the specimen by dropping it into boiling water for about half a minute. Spread out the body on a chip of balsa wood and pose it carefully, bracing and supporting it with pins as shown in Fig. 12. During the process of dehydration, the chip floats up-side-down on the surface of the liquid, a fact which must be taken into account when placing the pins. When the specimen is secure against drooping, even in an inverted position, fill a dish with 70% alcohol. Float the chip, face down, upon the surface. Put on the lid, and leave it over night.

On the following day, remove the float from the

bath and drain off all the alcohol you can by blotting gently with absorbent paper. Examine the specimen. If thin-skinned, it may have shrunk a little, so that the pins will have to be adjusted. Any alteration in posture must be made at this time. The specimen will be too stiff tomorrow.

Place the chip with its spider in a dish of 95% alcohol. After 24 hours the spider will be so rigid that you can safely remove all the pins but the few needed to fasten it to the wood. If those pins are short, the amount of liquid required to float the preparations will be materially reduced. Make sure there is no free alcohol clinging to the specimen, and transfer it to carbol-xylene. Unless the last trace of water is removed at this point, the specimen is likely to wrinkle when it dries, so leave it until it is perfectly clear, as near to transparent as its coloring will permit it to become. Then transfer to xylol, and rinse for 24 hours.

Unpin the specimen from the chip, drain it thoroughly, and set it in a warm, airy place to dry. If, in life, it was dark colored and hairy, like the jumping spiders, it will now be finished, ready to be glued into its place in the exhibit. If, like the crab-spiders, it was naked and brightly colored when alive, tint it with oil colors and allow it to dry for several days before fastening it into its permanent position.

This method of dry mounting can be applied, with greater or less success, to immature and adult insects, including the difficult hairy kinds, and to spiders, scorpions, phalangids, myriapods, and kindred creatures. It is more successful with tough, leathery species than with thin-skinned fleshy ones. The opacity which it imparts to naturally transluscent forms is a major drawback which can only occasionally be overcome by soaking the dried specimen in shellac or in a solution of paraffin in xylol.

Species of which it is not possible to produce satisfactory dry specimens for exhibition are best represented by life-size or enlarged models. If carefully made in the likeness of the living creatures, these are more lively in appearance than dry mummies, however painstakingly prepared.